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Ex vivo organotypic cerebellum slice culture (OCSC)

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An abbreviated version of this protocol was published in Life Science Alliance in Mar 2022

Spatial proteomics finds CD155 and Endophilin-A1 as mediators of growth and invasion in medulloblastoma

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Detailed protocol

Organotypic Cerebellum Slice Culture

Dissection: Wild type C57BL/6JRj mice pups are sacrificed at postnatal day (PND) 10–11 by decapitation under anesthesia. Cerebella are dissected and placed in cold Geys balanced salt solution containing kynurenic acid and glucose (GBSSK,*Falsig and Aguzzi Nat. Prot. 2008*). The correct dissection and separation of brain tissues are checked under a binocular loop.

Cerebellum slices: Then, the cerebella are embedded in 2% low melting point agarose gel. Solidified agarose blocks were glued onto the vibratome (VT 1200S, Leica, Wetzlar, Germany) disc with Roti Coll1 glue (0258.1 CarlRoth, Karlsruhe, Germany), mounted in the vibratome chamber filled with cold GBSSK and 350µm thick sections were cut (0.2mm/sec, 1.1mm). Slices are transferred to petri dishes filled with cold GBSSK. The correct section is checked under a binocular loop.

Deposit on inserts: Millipore inserts (PICM 03050, Merck Millipore, Burlington, VT, USA) are placed in six well plates filled with 1 mL cold brain slice culture medium (BSM) onto which the slices are then transferred using a Rotilabo-embryo spoon (TL85.1, Carl Roth GmbH, Karlsruhe, Germany). A maximum of three slices are placed per insert, and excess medium is removed.

Monitoring: Slices are monitored for any signs of apoptosis, and media is changed daily for two weeks.

Spheroid implantation: LA-EGFP expressing cells were seeded in 100µL in 96 low adhesion well plates (4520, Corning) for 48h, whose 24h with addition of 2µL per well of matrigel growth factor reduced (354230, Corning) if necessary to form tumor spheroids. Spheroids are filed on the slices (using 0.5µL pipette volume) and are incubated for 24h.

Treatments: Then, the co-cultures are treated for 5 days.

Immunofluorescence: Following the treatment, and in order to identify proliferative cells, the co-cultures are incubated with EdU from Click-iT® EdU Imaging kit (C10340, Invitrogen) following the protocol of the provider. For the fixation step, the co-cultures are fixed by putting 1mL of Formaldehyde 4% methanol free on the top and under each insert for 1h at room temperature, under agitation. After PFA fixation, the slices are cut and placed in a 24 well plate. Beforehand, the word “TOP” is written on the insert to be able to identify easily the slice side using black marker (VWR Lab Marker Fine Trip Alcohol/Waterproof Black 52877-310 VWR Scientific, San Francisco, CA). For the permeabilization step, the slices are incubated in standard cell culture trypsin EDTA and incubated at 37°C in a humidified incubator for 5 min. Then, they are incubated in phosphate buffered saline (PBS) containing 3% fetal bovine serum (FBS), 3% bovine serum albumin (BSA), and 0.3% Triton-x100 for 1h at room temperature on a shaker. We used an incubation time of 30min for the EdU solution and 45min for the revelation reagent mix buffer.

Primary antibodies are diluted in the blocking solution (BS; without Triton-x100) and incubated overnight on a shaker at 4°C. We used Anti-calbindin antibody [EP3478] (Rabbit monoclonal, 1:1000, ab108404, Abcam), Anti-Glial Fibrillary Acidic Protein (GFAP) antibody (Goat polyclonal, 2µg/mL, ab53554, Abcam), and Anti-Nuclei Antibody [clone 3E1.3] (Mouse monoclonal, 1:200, MAB4383, Merck).

Following three washes at RT using 5% BSA in PBS, secondary antibodies are diluted in BS and incubated for 3h at RT. We used Cy™3 conjugated AffiniPure Donkey Anti-Rabbit IgG (H+L) (1:200, #711-165-152), Brilliant Violet™421 conjugated AffiniPure Donkey Anti-Goat IgG (H+L) (1:100, #705-675 147), and Alexa™ Fluor 488 conjugated AffiniPure Donkey Anti-Mouse IgG (H+L) (1:200, #715-545-150) from Jackson Immuno Research.

Mounting: Finally, following three washes, the inserts are flat mounted in glycergel mounting medium (C0563, Dako, Jena, Germany).*(The side “TOP” is on the top.)*

Observation/acquisition: Four-color image acquisition is performed on an SP8 Leica confocal microscope (Leica Microsystems, Mannheim, Germany).

Solutions:

GBSS:

NaCl *(58.44 g/mol)* 8.00628 g/L
KCl *(74.5513 g/mol)* 0.37 g/L
Na_2HPO_4 *(141.96 g/mol)* 0.11 g/L
$CaCl_2.2H_2O$ *(147.01 g/mol)* 0.22 g/L
KH_2PO4 *(136.086 g/mol)* 0.09 g/L
$MgSO_4.6H_2O$ *(228.45 g/mol)* 0.06 g/L
$MgCl_2.6H2O$ *(203.3 g/mol)* 0.20 g/L
$NaHCO_3$*(84.007 g/mol)* 0.22 g/L
Store at 4 °C

GBSSK *(prepare just before use)*: 400 mL GBSS + 5.33 mL glucose 45 % + 4 mL kynurenic acid

Agarose 2%: 0.4 g / 20 mL GBSSK Keep at 37 °C

Brain Slice Medium BSM (100mL):

2x MEM *(8.046 g in 250 mL pH 7.4 filtered, 4 °C)* 25 mL BME 25 mL
Horse medium 25 mL 100x Glutamine 1 mL
Antibiotics 1 mL Glucose 45 % 1.375 mL
Water 21.625 mL pH 7.4, filtered, store at 4 °C

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Capdeville, C. and Baumgartner, M. (2022). Ex vivo organotypic cerebellum slice culture (OCSC). Bio-protocol Preprint. bio-protocol.org/prep1789.
2. Capdeville, C., Russo, L., Penton, D., Migliavacca, J., Zecevic, M., Gries, A., Neuhauss, S. C., Grotzer, M. A. and Baumgartner, M.(2022). Spatial proteomics finds CD155 and Endophilin-A1 as mediators of growth and invasion in medulloblastoma. Life Science Alliance 5(6). DOI: 10.26508/lsa.202201380